Gonadotrophin Stimulation Test of Ovarian Function*

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Brit. med. J., 1968, 1, 349-352

Dynamic tests of endocrine function have proved their value in the clinical study of the thyroid and adrenal glands, but their development for the assessment of gonadal function has been inconspicuous. Now that effective means of treating some disorders of ovarian function are available—even if at present only to a limited extent—the reliable determination of ovarian sensitivity to gonadotrophic stimulation has become a matter of practical clinical importance. This is particularly true when treatment with human gonadotrophic preparations is contemplated, not only in order to avoid the wastage of scarce material in cases which could not be expected to respond but also to minimize the real dangers of overstimulation of unduly sensitive ovaries (Mozes *et al.*, 1965). As has been shown by Crooke *et al.* (1966) the dosage of human follicle-stimulating hormone (F.S.H.) required to produce a positive ovarian response varies in different women over at least an eightfold range.

In woman with amenorrhoea it has long been customary to differentiate primary and secondary ovarian failure on the basis of the urinary gonadotrophin excretion, the mouse uterine weight response ("total gonadotrophin") being the basis of the test most widely used. An increased excretion is interpreted as an indication of intrinsic ovarian failure, the absence of the normal negative feed-back effect of ovarian oestrogen being the cause of excessive F.S.H. secretion by the pituitary gland; a low excretion is held to imply secondary failure, the ovary being inadequately stimulated by the deficient pituitary gonadotrophin production. Apart from the inherent defects of this bioassay procedure, which introduce a substantial degree of unreliability as well as inconvenience for general clinical use, determination of urinary gonadotrophin excretion fails to give any idea of whether ovaries which can respond to gonadotrophic stimulation are sensitive to such stimulation in greater or less degree. We doubt, indeed, whether increased urinary total gonadotrophin necessarily implies ovarian unresponsiveness, and we have good evidence that ovarian unresponsiveness may be found in some patients without increased urinary gonadotrophin excretion, as measured by the mouse uterine weight assay.

It was awareness of these needs and drawbacks which persuaded Shearman (1964) to propose the determination of the response of the urinary excretion of oestrone to injections of pregnant mares' serum gonadotrophin (P.M.S.) as a test of ovarian responsiveness to gonadotrophic stimulation in the human female. His brief report showed that in the 18 patients he studied intramuscular injections of 5,000 i.u. daily for three days produced significant rises in urinary oestrone excretion in 13 women with "normal" ovaries, no response in one patient with ovarian dysgenesis, and elevated responses in four women with polycystic ovaries. These findings suggest that human ovaries respond to P.M.S. in much the same way as they do to human pituitary or menopausal gonadotrophin. Because P.M.S. preparations are marketed, are readily available, and are much cheaper than human gonadotrophic material, their use for testing ovarian responsiveness would clearly be advantageous.

The procedure as carried out by Shearman (1964) involved the daily collection of 24-hour urine specimens for eight consecutive days, the first two providing a baseline, and the administration of three daily injections of gonadotrophin. This seemed to us to be too involved for routine clinical use on a wide scale. Moreover, it was not clear whether the peak of oestrone excretion had been reached within the time covered by Shearman's observations, and we therefore undertook a study with a view to evolving, if possible, a simpler procedure, the ultimate objective requiring no more than one 24-hour urine collection and a single gonadotrophin injection. In this we believe we have been successful.

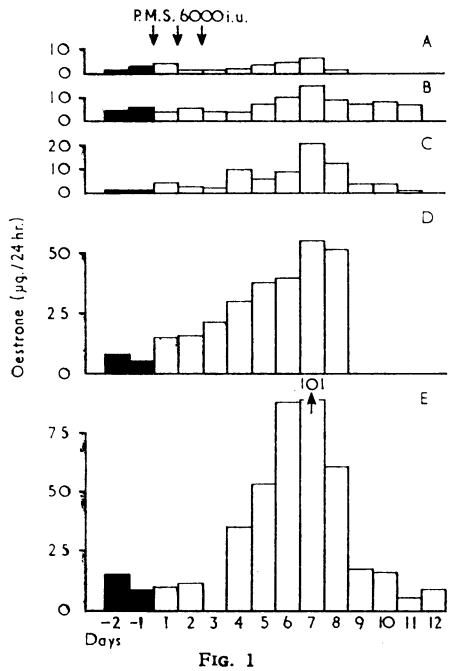

FIG. 1

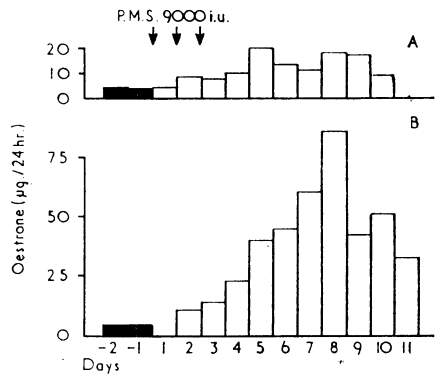

FIG. 2

FIG. 1.—Daily urinary oestrone excretion in five patients after three daily injections of P.M.S. 6,000 i.u. In this and Figs 2 to 5, days −1 and −2 are control days. FIG. 2.—Daily urine oestrone excretion in two patients after three daily injections of P.M.S. 9,000 i.u.

Methods

Studies have been made on 62 women (aged 17–36) who were patients attending the endocrine or fertility clinics at University College Hospital, or were seen privately by one of us (G. I. M. S.). Thirteen of these had primary amenorrhoea, all with chromatin-positive buccal smears, 34

* Part of a thesis to be presented by D. L. for the degree of Doctor of Philosophy in the University of London.

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had secondary amenorrhoea, and 15 had oligomenorrhoea. All patients had undergone a routine clinical assessment, but the extent of the special investigations varied considerably. In 44 patients gynaecography (pelvic radiography after induction of pneumoperitoneum) had been employed to visualize the ovaries and to estimate their size ; in three of these the result was unsatisfactory. In nine patients laparotomy had been performed, in four of them with wedge resection, before the present series of investigations were undertaken. Urinary total gonadotrophin excretion—mouse uterine weight assay after extraction by the method of Butt (1958)—has been determined by Miss P. M. Moritz on one of the baseline urine specimens in 41 patients.

Skin sensitivity testing was always done before intramuscular injections of P.M.S. were given. An intradermal injection of 0.1 ml. of a solution of P.M.S. 400 i.u. in 1 ml. of physiological saline was made on the ventral aspect of the forearm ; the appearance of a flare with pseudopodia within 15 minutes was regarded as positive and no patient showing such a response was given further P.M.S. Two patients (not among the above 62) were rejected on these grounds.

The plan initially followed was to collect two control 24-hour urine specimens to provide a baseline ; to give intramuscular injections of P.M.S. daily for three, two, or one day, the daily dose being 6,000, 9,000, 18,000, or 27,000 i.u. ; and to collect daily 24-hour urine specimens for from 6 to 12 days. The oestrone content of the urine was determined by the method of Lawrence (1968).

Results

Three injections of P.M.S. 6,000 i.u./day were given to five patients ; three of 9,000 i.u./day to two ; two of 9,000 i.u./day to four ; one injection of 18,000 i.u. was given to four ; and one of 27,000 i.u. to five patients. The results of these tests are shown in Figs. 1–5 respectively. It may be observed that the variation in the baseline determinations of oestrone excretion is small throughout the series as well as between replicates for any patient. Further, the pattern of oestrone excretion is remarkably similar, not only for a given dosage schedule but apparently for all the dosage schedules used. There is a rather clearly defined peak of oestrone excretion, and it is reached in every instance between days 6 and 8 after beginning injections. This is true whether one, two, or three injections were given.

In view of the above findings it seemed justifiable to employ only one baseline urine collection and a single injection followed

Urinary Oestrone Excretion on Day 6-7 or 7-8 After a Single Injection of P.M.S. in 42 Cases

Case No.	P.M.S. (I.U.)	Urinary Oestrone (μg./24 hr.)		
		Control	Days 6–7	Days 7–8
		Primary Amenorrhoea		
1	18,000	3·0		30·0
2	18,000	1·5	2·0	
6	18,000	4·5		3·0
7	18,000	2·0		16·5
8	18,000	2·5		106
10	18,000	3·0		5·4
11	27,000	3·0	5·5	
12	18,000	2·5		1·5
		Secondary Amenorrhoea		
14	18,000	7·1		112
15	18,000	11·5		15
16	18,000	1·5		40
17	18,000	7·5	567	
19	18,000	2·0	132	
20	18,000	4·0		41
21	18,000	2·5		18·5
23	18,000	3·0	6·5	
24	18,000	2·3		1·5
25	27,000	3·2		56
26	27,000	3·5		19·5
29	18,000	3·5		23
31	18,000	1·5		2·5
33	18,000	4·2		95
34	18,000	3·3	48	
36	18,000	10·0		415
37	18,000	3·0		11·2
38	18,000	6·5		555
39	18,000	3·5		1·5
40	18,000	2·3		6·6
41	18,000	3·0		2·5
42	27,000	3·5		4·8
47	18,000	3·4	9·1	
		Oligomenorrhoea		
48	18,000	5·0		45
49	18,000	7·0	465	
52	18,000	8·5		225
53	18,000	11·9		180
54	18,000	2·2		18
55	18,000	7·0		820
56	18,000	11·0		390
57	18,000	3·5		300
58	18,000	7·5	180	
60	18,000	4·0	14	
61	18,000	5·5		790

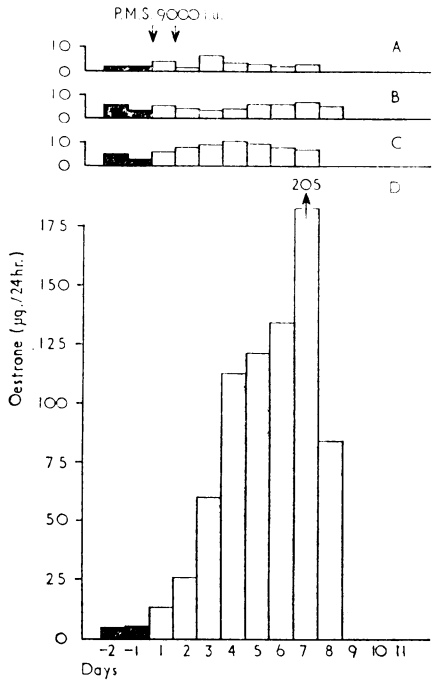

FIG. 3

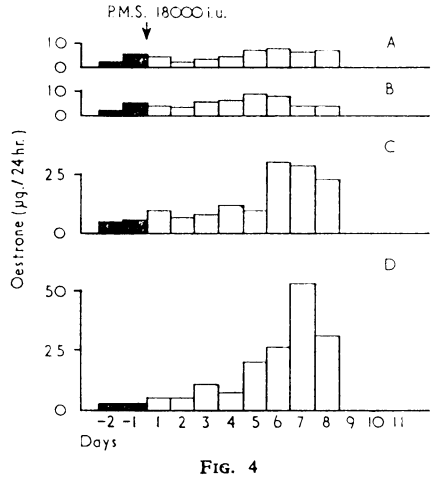

FIG. 4

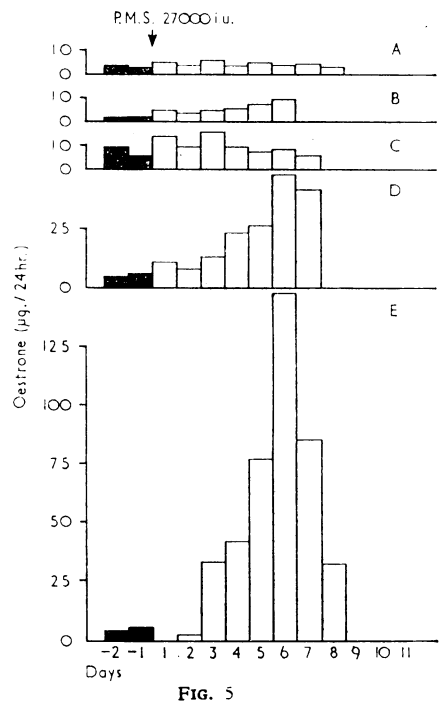

FIG. 5

FIG. 3.—Daily urinary oestrone excretion in four patients after two daily injections of P.M.S. 9,000 i.u. FIG. 4.—Daily urinary oestrone excretion in four patients after one injection of P.M.S. 18,000 i.u. FIG. 5.—Daily urinary oestrone excretion in five patients after one injection of P.M.S. 27,000 i.u.

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by urine collection on the sixth, seventh, or both days after the injection. Thirty-eight patients have thus been tested with P.M.S. 18,000 i.u. while four have received 27,000 i.u. (in every case given in a single injection). The results of these tests are shown in the Table.

Adverse Reactions

Six patients complained of symptoms after injection of P.M.S. One of these (Case 7) with primary amenorrhoea, who gave a normal response (16.5 μg. oestrone), experienced abdominal pain two days after the injection ; the pain lasted for a week, but pelvic examination gave no evidence of ovarian enlargement. The other five (Cases 36, 38, 52, 55, and 61) all had strong responses (225–820 μg. of oestrone). In three of these gynaecography had shown ovarian enlargement of some degree ; it had not been done in the other two. One patient (Case 38) developed typical findings of ovarian hyperstimulation (as reported by her general practitioner), but these subsided spontaneously. Subsequent laparotomy revealed typical polycystic ovaries. Case 52 was admitted as an acute abdominal emergency 14 days after the injection. Laparotomy revealed large bilateral theca-lutein cysts, one of which had ruptured. There was 1 pint (540 ml.) of cyst fluid free in the peritoneal cavity. Bilateral ovarian cystectomy was performed, with uncomplicated recovery. Two other patients complained of abdominal pain (one with dyspareunia) for one week after the injection. One patient (Case 55) complained of severe abdominal distension about one week after the injection, and was reported by her general practitioner to have ovarian enlargement. She had no pain, and the findings subsided spontaneously.

Discussion

Our findings confirm and, we believe, extend those of Shearman (1964). The peak of urinary oestrone excretion is reached most frequently on the seventh day after beginning P.M.S. injections (invariably, in our series, within days 6–8) and there seems to be no necessity to give more than a single injection. We believe 18,000 i.u. to be sufficient, though whether a smaller dose would be adequate has not been investigated. The range of variation of oestrone content among the baseline urine specimens is narrow enough to justify a single collection rather than two. The correlation, shown in Fig. 6, between the baseline and peak oestrone excretions is significant ($R=0.46$, $P<0.001$), but none the less knowledge of the baseline value adds little if anything to the information acquired by determining the oestrone response after P.M.S. injection. It seems doubtful, therefore, whether the determination of the baseline oestrone excretion is really necessary.

In order further to evaluate the gonadotrophin stimulation test we have compared the responses with the size of the ovaries, as judged by gynaecography, and with the urinary total gonadotrophin excretion. It must be admitted that the determination of ovarian size by gynaecography is only approximate, and we have therefore grouped our patients as having "small," "normal," or "large" ovaries, the allocation being based on past experience with the method checked by laparotomy observation of the ovaries. In Fig. 7 the peak excretion of oestrone (day 6 or 7) is shown for nine patients with gynaecographically small, 18 with normal, and 14 with large ovaries ; the differences of response in the three groups are statistically highly significant ($P<0.001$). One anomalous result is that marked with an asterisk in Fig. 7—namely, a weak response by a patient whose ovaries were enlarged on gynaecographic examination ; but this was done nine days after starting a course of clomiphene, and probably was a consequence of it.

The reliability of a single determination of urinary total gonadotrophin has been widely suspect, but, generally speaking, the finding of increased outputs has been accepted as indicative of lack of ovarian responsiveness even though the ability of the determination to discriminate between normal and subnormal outputs, implying normal or subnormal pituitary gonadotrophin production, is usually rejected. In Fig. 8 we have grouped the peak oestrone excretion (on day 6 or 7) for eight patients whose urinary total gonadotrophin was below the detectable level, for 13 excreting up to 20 units, for nine excreting 20 to 40 units ; and for 11 excreting 80 or more mouse uterine weight units per day. There is no significant difference in oestrone response even between the patients with no detectable gonadotrophins and those with an output of more than 80 units per day ($P>0.5$).

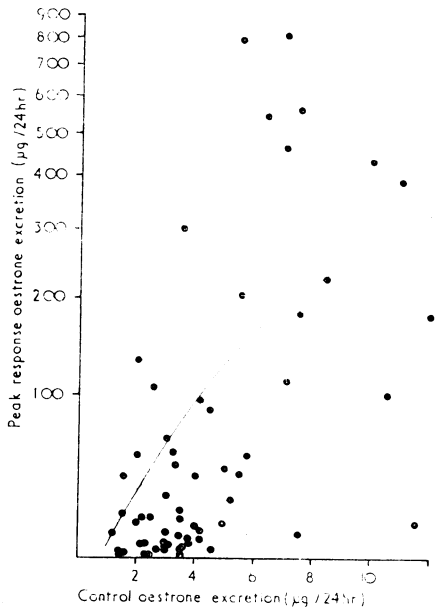

FIG. 6

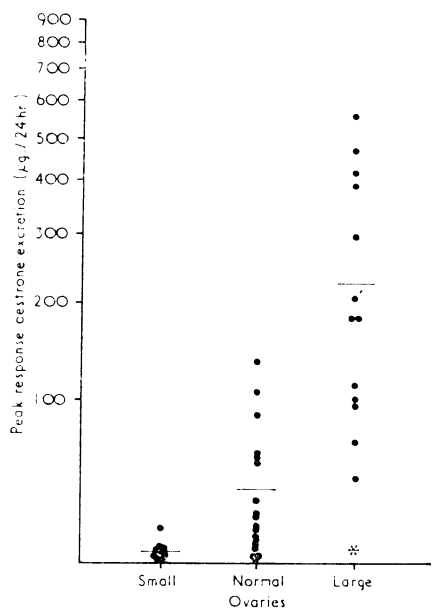

FIG. 7

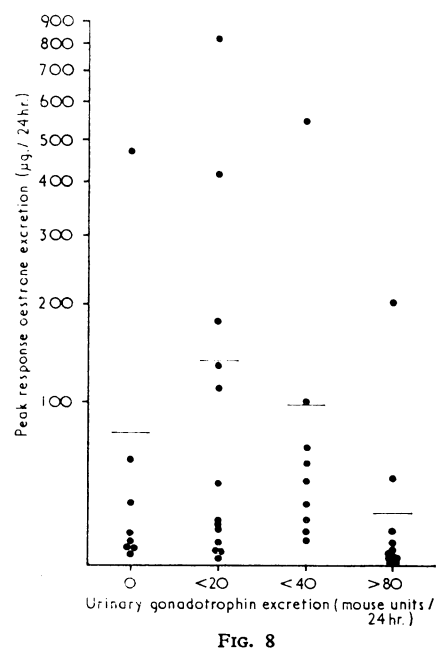

FIG. 8

FIG. 6.—Correlation of peak oestrone excretion in response to P.M.S. injection with control oestrone excretion before injection. The plot is of the equation $y=-25+29.6x$ ($R=0.46$, $P<0.001$). FIG. 7.—Peak oestrone excretion in response to P.M.S. injection grouped according to ovarian size as determined by gynaecography. The horizontal bars are the means for each group. For significance of the asterisk see text. Differences between groups highly significant ($P<0.001$). FIG. 8.—Peak oestrone excretion in response to P.M.S. injection grouped according to urinary "total gonadotrophin" excretion. Differences between groups not significant ($P>0.5$).

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From theoretical considerations one might have expected the first group to show a preponderance of strong responses with none at all in the latter. In fact, a strong response (205 μg. of oestrone per 24 hours) was given by a patient whose urinary total gonadotrophin excretion was 200 mouse uterine weight units per 24 hours. We believe these findings give firm support to Shearman's (1964) suggestion that the results of the gonadotrophin stimulation test would be more reliable than those of urinary gonadotrophin determination as usually employed for clinical diagnostic purposes.

Our observations lead us to suggest that a peak oestrone excretion after a single injection of 18,000 i.u. of P.M.S. of less than 15 μg./24 hours should be regarded as subnormal; that 15 to 80 μg./24 hours should be regarded as normal; and that responses in excess of 100 μg. imply ovarian hypersensitivity to P.M.S. We are not in a position to affirm that there would be similar responsiveness, or lack of it, to human gonadotrophin. However, at the upper and lower ends of the scale of response this certainly seems probable; in the intermediate range there might be room for doubt.

It is clear that there is a risk of producing ovarian hyperstimulation when a single injection of 18,000 i.u. of P.M.S. is given to a woman with polycystic ovaries. Our policy now, where the presence of such ovaries is thought possible, is to perform gynaecography before doing the gonadotrophin stimulation test and to withhold the latter if ovarian enlargement has been demonstrated.

Summary

After the injection of pregnant mares' serum gonadotrophin (P.M.S.) into women the urinary oestrone excretion reaches a peak most commonly on the sixth or seventh day. This is true if a total dose of 18,000 i.u. is given in a single injection or in two or three daily divided injections. On the basis of these observations a study has been made of a simple test of ovarian responsiveness to gonadotrophic stimulation involving a single injection of P.M.S. 18,000 i.u. and the determination of oestrone excretion on the seventh day after the injection (together with that in a baseline 24-hour urine specimen before treatment, shown not to be really necessary). The ovarian response so determined correlated well with ovarian size as estimated by gynaecography, while in contrast the urinary "total gonadotrophin" excretion was shown to be an unreliable guide to ovarian gonadotrophin responsiveness. It is suggested that a peak oestrone excretion after a single injection of 18,000 i.u. of P.M.S. of less than 15 μg./24 hours indicates a subnormal ovarian response; that 15–80 μg. covers the normal range; and that more than 100 μg. implies ovarian hypersensitivity, most probably indicative of polycystic ovaries.

Of 62 women investigated, none of whom had given a positive intradermal reaction, six complained of symptoms after the injection of P.M.S. In four these were minor, but two women developed more serious signs and symptoms of ovarian hyperstimulation, one being admitted as an abdominal surgical emergency. Both these last two women had polycystic ovaries, and use of the test is therefore not recommended in women *known* to have such ovaries.

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Comparison of Continuous and Intermittent Anorectic Therapy in Obesity

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Brit. med. J., 1968, **1**, 352–354

Obese outpatients treated initially with diet and an anorexigenic drug usually lose more weight than those treated by diet alone. It is generally stated, however, that in most patients the anorectic loses its effect after two to four months of administration, and for this reason repeated short courses of drug treatment have been advocated (Silverstone, 1967). We report the results of a double-blind 36-week study undertaken to determine the efficacy of continuous and intermittent therapy with the anorexiant phentermine (Duromine).

Material and Methods

One hundred and eight women aged from 21 to 60 were included in the trial at the time of their first referral to the department. All were clinically obese and overweight by at least 20% of their standard (U.S.A. Medico-Actuarial Investigation, 1912). None had evidence of endocrine or cardiovascular disease, and patients who had previously experienced troublesome side-effects to amphetamine or its derivatives or who were thought to be psychologically unsuitable were excluded. No patient had knowingly taken an anorectic agent at any time during the previous two years, and though many stated that they were "dieting" none was on a prescribed dietary regimen.

Each patient was initially weighed, examined, had a dietary history taken, and was allocated to one of three comparable groups, each comprising 36 patients. Those in the first group were given four weeks' supply of dummy capsules, those in the second group were given capsules of identical appearance containing 30 mg. of phentermine, and patients in the third group were given alternate four-week supplies of the active and dummy capsules. They were told to take one capsule daily before breakfast, as phentermine is a drug-resinate complex which need be taken only once daily. Phentermine is also available as capsules containing 15 mg. of active drug. All patients were instructed in a diet based on the principles of simple carbohydrate restriction and designed to provide approximately 1,000 calories daily. No dietary advice was thereafter given.

Patients were asked to attend a special clinic every four weeks, wearing as nearly as possible the same clothing. Those who failed to report within a week of their appointment were withdrawn from the trial (Table I). At each visit the patient was weighed and was asked if any symptoms had occurred that she attributed to the capsules. She was then given a further four weeks' supply of capsules, the nature of which was not known

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